Effects of Corydalis Tuber on the Thyroid Function in Rats

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ABSTRACT

The effects of Corydalis tuber extracts on the thyroid function of rats were investigated in this study. The methanol, *n*-butanol, water, and chloroform extracts of Corydalis tubers significantly decreased thyroid function in hyperthyroid rats after 14 days of treatment. The chloroform B extract also possessed antithyroid activity, similar to the effects of propylthiouracil (PTU). Corydalis tuber extracts did not influence TSH level on thyroid and pituitary weight in normal rats whereas PTU increased thyroid gland weight. The chloroform B extract of Corydalis tubers decreased TRH-induced TSH levels in hyperthyroid rats after 20 days of treatment whereas PTU increased TSH levels in hyperthyroid rats. These results suggest that (±)-tetrahydropalmatine (*dl*-THP), a protoberberine-type alkaloid isolated from the chloroform B extract of Corydalis tubers, is the active ingredient of Corydalis yunhusuo. The mechanism of action may be related to inhibition of TSH secretion in the pituitary.

Key words: Corydalis tuber; Thyroid function; Hyperthyroidism; TSH secretion.

INTRODUCTION

The thyroid is a major endocrine gland for maintaining the equilibrium of human metabolism. Phenomena such as nervous tension, dietary imbalance, environmental pollution, misuses of drugs and disease infection may cause abnormal function of the thyroid.[1] The conventional methods for treating thyroid symptoms include surgery, radiation with radioactive isotopes and treatment with thyroid inhibitors.[2]

Hyperthyroidism is often associated with aberrations in the regulation of the hypothalamus-pituitary-thyroid (HPT) system. Typical characteristics include elevated levels of T3, T4, FT3 and FT4.[3] The conventional antithyroid drug, propylthiouracil (PTU), inhibits the formation of thyroid hormones by interfering with the incorporation of iodine into tyrosyl residues of thyroglobulin; in addition, it also inhibits the coupling of these iodotyrosyl residues to form iodothyronines.[4,5] However, PTU stimulates the thyrotropine-stimulating hormone (TSH) through feedback inhibition which can enlarge follicle cells, cause loss of colloid and decrease blood cell numbers.[6,7] Therefore, the discovery of new anti-thyroid drugs with different modes of action from PTU is worthwhile.

Corydalis tuber, the tuber of Corydalis yunhusuo, was first described in Kai-Pao-Pen-T'sao. It possesses analgesic, sedative and anti-thyroid effects.[8] In our previous study, the crude extract of Corydalis tuber was found to posses anti-hyperthyroid activity in rats.[9] Therefore, we partitioned the crude extract with different organic solvents, and produced four organic extracts (methanol, *n*-butanol, water and chloroform B). In this study, the thyroid gland, pituitary and cortex weight were measured to investigate the side effects of each Corydalis tuber extract. Secondly, the T3, T4, FT3, FT4 and TSH levels of normal and L-T4-induced hyperthyroid rats were also measured to investigate the effects of each Corydalis tuber extract on thyroid function. Finally, measurements were also taken of the TSH levels induced by thyroid-releasing hormone (TRH) in normal and L-T4-induced hyperthyroid rats to assess the possible mode of action of each Corydalis tuber extract.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing between 250-280 gm were housed individually under conditions of controlled temperature (22 °C), humidity (55 ± 5 %) and a 12 h light-dark cycle (lights on from 07:00-19:00). The animals were allowed free access to water and standard laboratory meals.

Drug Preparations

Corydalis tuber extracts [methanol (CM), *n*-butanol (CB), water (CW) and chloroform B (CCB)] were prepared as previously described.[8] PTU, administered orally at 5.0 mg/kg, served as a positive control group in each experiment. Rats received subcutaneous injections of 300 μg/kg L-T4 to induce hyperthyroidism. TRH was administered intraperitoneally at 300 μg/kg to stimulate the release of TSH. The RIA kits were supplied by the Abbote Co. Ltd (Chicago, USA).

Hemodynamic Variables in Normal Rats

Normal rats were treated with Corydalis tuber extract at a daily dose of 50.0 mg/kg or *dl*-tetrahydropalmatine (*dl*-THP) at a daily dose of 2.5, 5.0, 10.0 mg/kg for 14 days. On the 15th day, 50 mg/kg pentobarbital was intraperitoneally injected into the rats. The rat abdominal cavity was incised. Arterial blood was collected for 2 hours at room temperature and centrifuged at 2500 rpm for 10 minutes to collect serum. The effectiveness of these treatments was assessed in 6 rats in each group by comparing TSH levels, concentrations of serum T3 (triiodothyroxine), serum T4 (thyroxin), free T3 and free T4, and the final thyroid, pituitary and cortex weights.[6] The concentrations of TSH, T3 (triiodothyroxine), T4 (thyroxin), free T3 and free T4 in serum were finally measured by RIA.[3,10]

Hemodynamic Variables in L-T4-induced Hpyerthyroid Rats

Rats were subcutaneously injected for 12 days with a daily dose of 300 μg/kg L-T4 to induce hyperthyroidism, and then treated with Corydalis tuber extracts at a daily dose of 50.0 mg/kg, PTU at a daily dose of 5.0 mg/kg orally or *dl*-tetrahydropalmatine (*dl*-THP) at a daily dose of 2.5, 5.0, 10.0 mg/kg for 14 days. On the 15th day, 50 mg/kg pentobarbital was intraperitoneally injected into the rats. The rat abdominal cavity was incised, arterial blood was collected and serum was recovered by settling at room temperature for 2 hrs and centrifuged at 2500 rpm for 10 minutes. The effectiveness of these treatments was assessed in 6 rats in each group by comparing TSH levels, concentrations of serum T3 (triiodothyroxine), serum T4 (thyroxin), free T3 and free T4, and the final thyroid, pituitary and cortex weights. The concentrations of TSH, T3 (triiodothyroxine), T4 (thyroxin), free T3 and free T4 in serum sample were measured by RIA.

Effect of Chloroform B Extract of Corydails Tubers (CCB) on TRH-induced TSH Levels in Rats

Normal group

Rats were continuously treated with CCB at a daily dose of 50.0 mg/kg p.o. for 20 days. On the 21th day, 300 μg/kg TRH was intraperitoneally injected into the rats. Rat abdominal cavities were incised and arterial blood was collected. Serum was recovered and the concentration of TSH in the serum was measured by RIA.[11]

L-T4 induced hyperthyroid group

Rats were subcutaneously injected for 12 days with a daily dose of 300 μg/kg L-T4 to induce hyperthyroidism. Rats then received for a daily p.o. dose of 50.0 mg/kg CCB for 20 days. On the 21th day, 300 μg/kg TRH was intraperitoneally injected into the rats. Rat abdominal cavities were incised, arterial blood was collected and concentration of TSH in the

serum was measured by RIA.

RESULTS

Effects of Corydalis Tuber Extracts on Thyroid Function in Rats

Normal group

As shown in Table 1, treatment with 50 mg/kg of CM, CW or CCB extract of Corydalis tubers for 14 days remarkably reduced FT4 concentrations in rat serum. The CCB extract also remarkably reduced FT3 concentrations in rat serum. The CB extract, however, did not affect the concentrations of FT3 and FT4 in rat serum.

L-T4 induced hyperthyroid group

As shown in Table 2, treatment with 50 mg/kg of CM, CW or CCB extract of Corydalis tubers for 14 days remarkably reduced serum T3 ($p < 0.05$-0.01), T4 ($p < 0.05$- 0.01), FT3 ($p < 0.01$-0.001) and FT4 ($p < 0.05$-0.001) concentrations in L-T4-induced hyperthyroid rats. However, none of the Corydalis tuber extracts influenced TSH values. PTU remarkably reduced serum T3 ($p < 0.001$), T4 ($p < 0.001$), FT3 ($p < 0.01$) and FT4 ($p < 0.001$), but increased TSH ($p < 0.001$) concentrations in L-T4-induced hyperthyroid rats.

Effects of Corydalis Tuber Extracts on Thyroid, Pituitary and Cortex Weights in Rats

As shown in Tables 3, treatment with 50.0 mg/kg of Corydlis tuber extracts for 14 days did not significantly change thyroid weight in comparison with the normal group. However, rats treated with PTU clearly exhibited an increase in thyroid weight ($P < 0.001$).

Table 1. Effects of Corydalis Tuber Extracts on Thyroid Function in Normal Rats

Drugs	TSH (μu/mL)	T3 (ng/dL)	T4 (μg/dL)	FT3 (ng/dL)	FT4 (ng/dL)
Control (saline)	2.63±0.38	39.77±6.01	2.21±0.21	1.15±0.09	1.94±0.29
CM (50 mg/kg)	2.60±0.46	38.20±3.16	1.88±0.37	0.85±0.37	1.36±0.21*
CB (50 mg/kg)	2.63±0.34	43.35±3.76	2.34±0.4	0.98±0.33	1.72±0.27
CW (50 mg/kg)	2.81±0.41	43.37±8.25	2.10±0.34	1.02±0.24	1.34±0.32*
CCB (50 mg/kg)	2.19±0.34	38.18±4.63	1.98±0.28	0.80±0.17*	1.29±0.29*

Data are presented as the mean±SEM (n = 6). CM: methanol extract of Corydalis tubers.
CB: *n*-butanol extract of Corydalis tubers. CW: water extract of Corydalis tubers.
CCB: chloroform B extract of Corydalis tubers.
Significantly different from control group (*$p < 0.05$).

Table 2. Effects of Corydalis Tuber Extracts on Thyroid Function in L-T4-induced Hyperthyroid Rats.

Drugs	TSH (μU/mL)	T3 (ng/dL)	T4 (μg/dL)	FT3 (ng/dL)	FT4 (ng/dL)
HP (saline)	1.01±0.10	63.94±14.28	4.68±0.78	4.15±0.10	6.42±1.42
HP+CM (50 mg/kg)	1.03±0.07	39.93±4.74*	2.85±0.35*	2.48±0.50**	2.88±0.48**
HP+CB (50 mg/kg)	1.12±0.10	49.90±8.9*	2.87±0.68*	2.47±0.26**	3.68±0.90**
HP+CW (50 mg/kg)	1.20±0.19	42.40±4.3**	2.97±0.78*	2.44±0.36**	3.87±0.89**
HP+CCB (50 mg/kg)	1.08±0.28	50.90±1.2*	2.57±0.49**	1.80±0.37***	2.58±0.46**
HP+PTU (5 mg/kg)	3.98±0.40***	17.92±1.43***	1.11±0.02***	0.44±0.02***	0.47±0.04***

Data are presented as the mean±SEM (n = 6). CM: methanol extract of Corydalis tubers.
CB: *n*-butanol extract of Corydalis tubers. CW: water extract of Corydalis tubers.
CCB: chloroform B extract of Corydalis tubers.
HP: Hyperthyroidism induced by daily i.p. injection of 300 μg/kg L-T4, s.c. for 12 days.
PTU: Propylthiouracil p.o. for 14 days.
Significantly different from HP group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Table 3. Effects of Corydalis Tuber Extracts on Rat Thyroid, Pituitary and Cortex Weights in Normal Rats

Drugs	B.W. (g)	Thyroid weight (mg/100 g BW)	Pituitary weight (mg/100 g BW)	Cortex weight (mg/ 100 g BW)
Control	246±14	8.0±1.2	18.9±3.0	24.2±4.4
CM (50 mg/kg)	272±9	7.3±1.5	16.8±1.0	27.9±2.7
CB (50 mg/kg)	262±15	8.4±1.0	17.4±2.8	26.2±4.9
CW (50 mg/kg)	257±15	7.6±0.4	17.9±2.7	24.1±3.6
CCB (50 mg/kg)	264±6	8.4±1.3	17.5±2.8	26.7±4.8
PTU (5 mg/kg)	261±10	26.8±2.5***	20.8±2.5	27.1±3.8

Data are presented as the mean±SEM (n = 6). CM: methanol extract of Corydalis tubers.
CB: *n*-butanol extract of Corydalis tubers. CW: water extract of Corydalis tubers.
CCB: chloroform B extract of Corydalis tubers. B.W.: Body weight.
PTU: Propylthiouracil p.o. for 10 days.
Significantly different from control group (***$p < 0.001$).

Effects of Chloroform B Extract of Corydalis Tubers (CCB) on TRH-induced TSH Levels in Rats

As shown in Fig. 1, treatment with 50.0 mg/kg CCB daily for 14 days remarkably reduced the serum concentration of TSH in the L-T4-induced hyperthyroid rats ($p < 0.05$). In

contrast, PTU increased the serum concentration of TSH in the L-T4-induced hyperthyroid rats ($p < 0.01$).

Effects of *dl*-Tetrahydropalmatine (*dl*-THP) on Thyroid Function in Rats

Normal group

As shown in Table 4, treatment with 2.5, 5.0 or 10.0 mg/kg *dl*-THP for 14 days did not affect the concentrations of TSH, T3, T4, FT3 and FT4 in normal rats. PTU, on the other hand, remarkably decreased the serum concentration of T3 ($p < 0.001$), T4 ($p < 0.001$), FT3 ($p < 0.001$) and FT4 ($p < 0.001$) and increased TSH concentration ($p < 0.001$) in normal rats.

L-T4 induced hyperthyroid group

As shown in Table 5, treatment with 2.5, 5.0 or 10.0 mg/kg *dl*-THP for 14 days remarkably decreased serum T3, T4, FT3 and FT4 concentrations in L-T4-induced hyperthyroid rats. PTU (5.0 mg/kg) also reduced the serum T3 ($p < 0.001$), T4 ($p < 0.001$), FT3 ($p <$

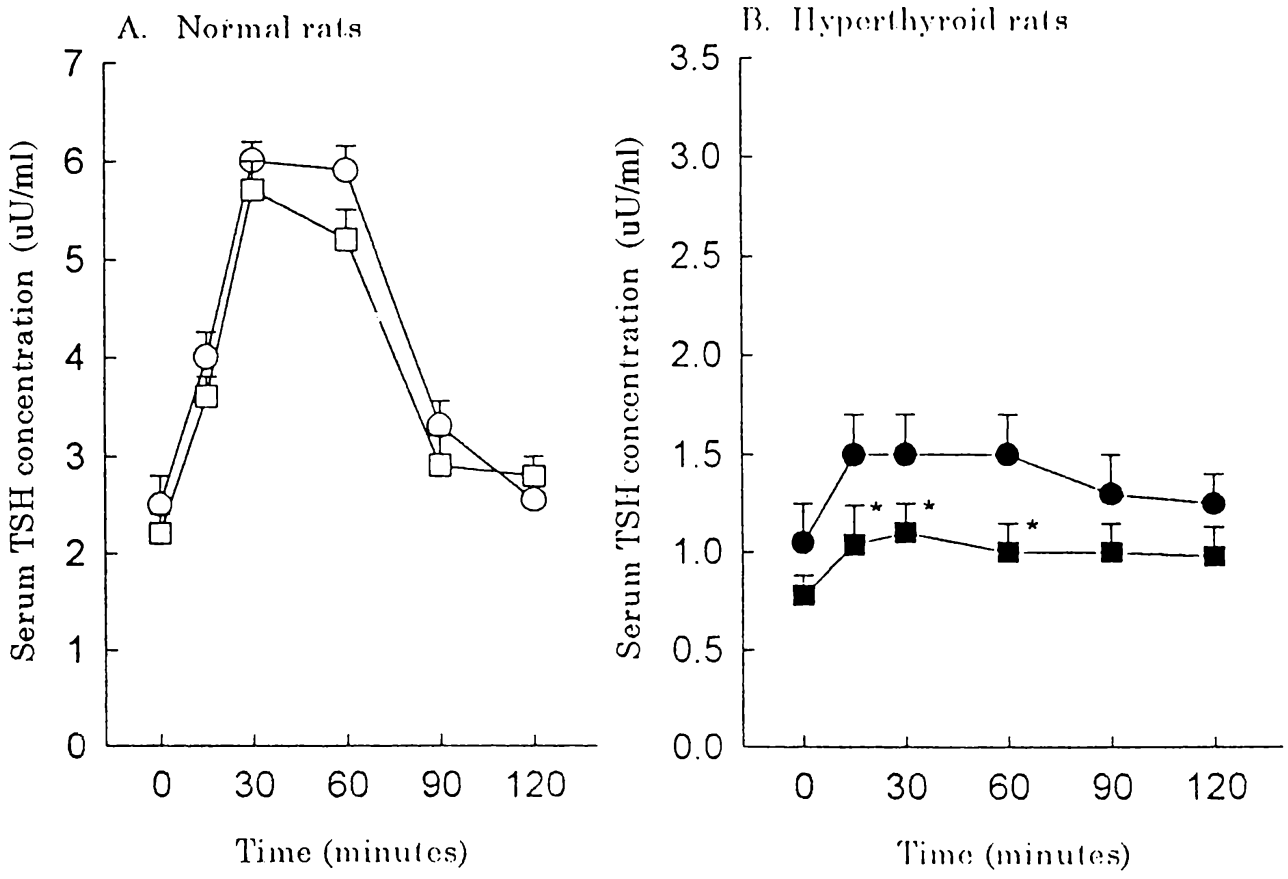

Fig. 1. Effects of Chloroform B Extract of Corydalis Tubers on TRH-induced TSH Levels in Rats. (A) Normal rats. Data are presented as the mean ±SEM of 6 rats. O–O: Vehicle. □–□: 50 mg/kg Chloroform B Corydalis tuber extracts for 20 days. (B) Hyperthyroid rats induced by 300 μg/kg L-T4 s.c. for 12 days. Data are presented as the mean ±SEM of 5 rats. ●–●: Vehicle. ■–■: 50 mg/kg Chloroform B Corydalis tuber extracts for 20 days. Significantly different from hyperthyroid rats (*$p < 0.05$).

Table 4. Effects of *dl*-Tetrahydropalmatine (*dl*-THP) on Thyroid Function in Normal Rats

Drugs	Dose (mg/kg)	TSH (μU/mL)	T3 (ng/dL)	T4 (μg/dL)	FT3 (ng/dL)	FT4 (ng/dL)
Control	saline	2.3±0.2	54.1±3.4	2.7±0.2	1.2±0.1	2.0±0.2
dl-THP	2.5	1.9±0.1	56.8±3.1	2.3±0.5	1.3±0.1	1.7±0.5
dl-THP	5.0	2.5±0.4	58.8±3.7	2.5±0.2	1.2±0.2	2.1±0.2
dl-THP	10.0	1.9±0.1	49.1±2.5	2.3±0.3	1.2±0.1	1.9±1.5
PTU	5.0	4.5±0.4***	31.8±3.1**	0.9±0.1***	0.4±0.1***	0.4±0.1**

Data are presented as the mean±SEM (n = 6). *dl*-THP: *dl*-Tetrahydropalmatine.
PTU: Propylthiouracil.
Significantly different from control group (**p < 0.01, ***p < 0.001).

Table 5. Effects of *dl*-THP on Thyroid Function in L-T4-induced Hyperthyroid Rats

Drugs	Dose (mg/kg)	TSH (μU/mL)	T3 (ng/dL)	T4 (μg/dL)	FT3 (ng/dL)	FT4 (ng/dL)
Control	saline	2.3±0.2	54.1±3.4	2.7±0.2	1.2±0.1	2.0±0.2
HP	-	1.0±0.1	72.8±8.9	6.6±0.5	4.1±0.5	9.1±0.9
HP+THP	2.5	0.9±0.1	49.9±8.9*	5.1±0.1*	1.8±0.6*	5.7±0.9**
HP+THP	5.0	0.8±0.1	42.4±4.3**	4.9±0.2*	1.4±0.6**	5.7±0.9**
HP+THP	10.0	0.9±0.1	50.9±1.2*	5.1±0.2*	1.5±0.6**	5.3±1.1**
HP+PTU	5.0	4.0±0.5***	30.3±3.3***	2.0±0.3***	1.1±0.3***	3.2±0.9***

Data are presented as the mean±SEM (n = 6)
HP: Hyperthyroidism induced by 300 μg/kg L-T4, s.c. for 12 days.
dl-THP: *dl*-Tetrahydropalmatine.
PTU: Propylthiouracil.
Significantly different from control group (*p < 0.05, **p < 0.01, ***p < 0.001).

0.001), FT4 (p < 0.001) concentrations, but increased TSH (p < 0.001) concentration in L-T4-induced hyperthyroid rats.

DISCUSSION

Hyperthyroidism is related to imbalances of thyroid hormone regulation. Thioureylene drugs, e.g., propylthiouracil (PTU) and methimazole, are extensively employed in the treatment of Graves' hyperthyroidism. They act similarly on the thyroid to block thyroid peroxidase-catalyzed iodination and coupling.[2,12] Furthermore, they in-

crease TSH levels through feedback stimulation to cause enlargement of thyroid cells.

The effects of Corydalis tuber extracts on serum T3, T4, FT3, FT4 and TSH concentrations in normal and hyperthyroid rats were investigated. All Corydalis tuber extracts significantly decreased T3, T4, FT3 and FT4 levels in hyperthyroid rats. The chloroform B Corydalis tuber extract exhibited the best antithyroid effect. The chloroform B Corydalis tuber extract did not influence TSH levels in normal and hyperthyroid rats. PTU also decreased T3, T4, FT3 and FT4 levels in normal and hyperthyroid rats. PTU, however, increased TSH levels in normal and hyperthyroid rats. Therefore, Corydalis tuber extracts and PTU similarly affected thyroid function in rats. Low doses of antithyroid drugs induce hypothyroidism characterized by increased TSH levels and decreased serum T3 or T4 levels.[13] The above results indicate that Corydalis tuber extracts and PTU decreased thyroid function. However, the mode of action of the Corydalis tuber extracts are different from that of PTU.

PTU can decrease the thyroid function and increase TSH levels through feedback stimulation to cause enlargement of thyroid cells. Therefore, the effects of Corydalis tuber extracts on the thyroid, pituitary and cortex weights were also investigated. Corydalis tuber extracts did not affect thyroid, pituitary and cortex weights in normal and hyperthyroid rats. However, PTU remarkably increased thyroid weight in normal and hyperthyroid rats. These results indicate that Corydalis tuber extracts can decrease thyroid function without feedback stimulation of TSH levels.

The mode of action of the chloroform B extract of Corydalis tubers was also investigated in the TRH-stimulation test. Inhibition of thyrotropin (TSH) was evaluated by determining serum TSH in respone to thyrotropin-releasing hormone (TRH) in normal subjects.[14] Neither the chloroform B extract of Corydalis tubers nor PTU influenced TSH levels induced by TRH in normal rats. The chloroform B extract decreased the TRH-induced TSH levels in hyperthyroid rats whereas PTU increased the TRH-induced TSH levels. These results indicate that the mode of action of the chloroform B extract of Corydalis tubers on hyperthyroidism may be related to the inhibition of TSH secretion in the pituitary. This is in contrast to PTU which inhibits the formation of thyroxin hormones in the thyroid gland.

In a recent study, five protoberberine-typed alkaloids, including C-B1((±)-tetrahydropalmatine; *dl*-THP), C-B2 ((+)-corydaline), C-B3 ((±)-tetrahydrocoptisine), C-B4 ((-)-tetrahydrojatrorrhizine) and C-B5 ((±)-palmatine), were isolated from the chloroform B extract of Corydalis tubers. Recent studies have demonstrated that (±)-palmatine and (±)-tetrahydropalmatine (*dl*-THP) possess marked sedative and analgesic effects.[8] Furthermore, (±)-tetrahydropalmatine (*dl*-THP) has antithyroid activity (see Tab (4), Tab (5)). (±)-Tetrahydropalmatine (*dl*-THP), a protoberberine-type alkaloid, isolated from the chloroform B extract of Corydalis tubers, may be an active ingredient of Corydalis tuber. Further

studies are necessary to determine the antithyroid mechanism and toxicity of (±)-tetrahydropalmatine.

In conclusion, Corydalis tuber extracts did not influence T3, T4, FT3, FT4 and TSH concentrations in normal rats, indicating that each extract did not influence thyroid function in normal rats. Corydalis tuber extracts decreased T3, T4, FT3, FT4 levels in L-T4-induced hyperthyroid rats. The chloroform B extract of Corydalis tubers had the best antithyroid activity. The chloroform B extract of Corydalis tubers decreased TRH-induced TSH levels in L-T4-induced hyperthyroid rats. These results suggest that the antithyroid effect of the chloroform B extract of Corydalis tubers may be related to the inhibition of TSH secretion in the pituitary. (±)-Tetrahydropalmatine (*dl*-THP), a protoberberine-type alkaloid isolated from the chloroform B extract of Corydalis tuber, may be an active ingredient of Corydalis tubers.

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REFERENCES

1. Daughaday, W. H.; Herington, A. C.; Philips, L. S. The Regulation of Growth by Endocrine. *Ann. Rew. Physiol.* **1975**, *37*, 211-218.
2. Singer, P. A. Thyroid Function Tests and Effects of Drugs on Thyroid Function in Mannual of Endocrinology and Metabolism. Lavin, N. Ed.; Little Bromn and Company, Boston 1988; pp 341-345.
3. Walker, P.; Coulombe, P.; Dussault, H. Effects of Neonatal Hyperthyroidism of The Development of The Hypothalamic-Pituitary-Thyroid Axis in The Rat. *Endocrinology* **1982**, *100*, 1037-1047.
4. Davidson, S.; Soodak, M.; Neary, J. T. The Irreversible Inactivation of Thyroid Peroxidase by Methymercoptoimmidazole, Thiouracil, and Propylthiouracil in Vitro and Its Relationship to in Vivo Findings. *Endocrinology* **1978**, *103*, 871-875.
5. Morley, J. E. Neuroendocrine Control of Thyrotropin Secretion in Endocrine Reviews. Morley, J. E. Ed.; Churchill. Livingstone, London, 1981; pp 396-436.
6. Taurog, A. The Mechanism of Action of The Thiouleylen Antithyroid Drugs. *Endocrinology* **1976**, *98*, 1031-1036.
7. Amrheim, J. A.; Kenny, F. M.; Ross, D. Granulocytopenia, Lupus-like Syndrome and Other Complications of Propylthiouracil Therapy. *J. Pediat.* **1970**, *76*, 54-63.
8. Hsieh, M. T.; Hung, H. J.; Tsai, H. Y. and Chiu, N. Y. The Effects of The Bioactive Con-

stituents of Corydalis Tuber on The Central Nervous System. *Chin. Pharm. J.* **1989**, *41*, 227-236.

9. Hsieh, M. T.; Lin, C. N.; Tsai, H. Y.; Hung, C. F. The Effects of Yanhusuo on Thyroid Function and Fetal Growth in Pregnant Rats. *Chi. Med. Col. Ann. Bul.* **1989**, *15*, 81-197.
10. Snyder, P. J.; Utiger, R. D. Inhibition of Thyrotropin Response to Thyrotropin-Releasing Hormone by Small Qualities of Thyroid Hormones. *J. Clin. Invest.* **1972**, *51*, 2077-2084.
11. Sawin, C. T.; Hershman, J. M. Clinical Use of Thyrotropin-releasing Hormone. *Pharmacol. Ther.* **1976**, *1*, 351-366.
12. Engler, H.; Taurog, A.; Luthy, C.; Dorris, M. L. Reversible and Irreversible Inhibition of Thyroid Peroxidase-catalyzed Iodination by Thioureylene Drugs. *Endocrinology* **1983**, *112*, 86-95.
13. Mannisto, P. T.; Ranta, T.; Leppaluoto, J. Effects of Methylmercaptoimidazole (MMI), Propylthiouracil (PTU), Potassium Perchlorate ($KClO_4$) and Potassium Iodide (KI) on The Serum Concentrations of Thyrotropin (TSH) and Thyroid Hormones in The Rat. *Acta. Endocrinol. (Copenh)* **1979**, *91*, 271-281.
14. Anderson, M. S.; Bowers, C. Y.; Kastin, A. J.; Schalch, D. S.; Schally, A. V.; Snyder, P. J.; Utiger, R. D.; Wilber, J. F.; Wise, A. J. Synthetic Thyrotropin-releasing Hormone: A Potent Stimulator of Thyrotropin Secretion in Man. *N. Engl. J. Med.* **1971**, *285*, 1279-1283.